2761-Plat
Dynamics of C-Terminal Gtα and Gsα Peptides in the Binding Cavity of Active GPCRs
Alexander S. Rose[1], Ulrich Zachariae[2], Patrick Scheerer[1], Helmut Grubmüller[3], Klaus P. Hofmann[1], Peter W. Hildebrand[1].
[1]Charité – Universitätsmedizin Berlin, Berlin, Germany, [2]University of Edinburgh, Edinburgh, United Kingdom, [3]Max-Planck-Institute for Biophysical Chemistry, Göttingen, Germany.
On activation by agonists, G protein coupled receptors (GPCRs) transmit extracellular signals into the cell by catalyzing GDP/GTP exchange in heterotrimeric G proteins (Gαßγ). During this reaction the Gα C-terminus (GαCT) that binds to an open binding cavity of active GPCRs is displaced. We report on molecular dynamics simulations of GsαCT and GtαCT peptides interacting with the active GPCRs rhodopsin and β2-adrenoceptor, respectively. Starting from their X-ray structure position, all specific interactions are preserved. When starting from the position in a GDP bound intermediate, obtained from flexible docking, GαCT switches within nanoseconds to the X-ray structure position. Both GαCTs are thereby rotated and expose two distinct sites to highly conserved motifs at R3.50 and at the P3.57 cap of TM3. Our analysis highlights the role of GαCT as an active agent in transferring the signal from the receptor/ G protein interface to the Gα nucleotide binding site.

2762-Plat
Unraveling Allostery with Simulations of Rhodopsin Photocycle Intermediates
Nicholas Leioatts, Tod D. Romo, Alan Grossfield.
University of Rochester, Rochester, NY, USA.
G protein-coupled receptors (GPCRs) are a biomedically important class of membrane proteins, accounting for about one third of all FDA approved drugs. They act as molecular transducers, allosterically passing signals across the cell membrane. This allosteric modulation of GPCR signal is vital to their pertinence as drug targets, but the details of the mechanism are not fully understood. Two prominent hypotheses exist to describe how ligands affect changes in signaling. In the induced fit mechanism, the ligand is predicted to drive the protein to a new conformation. Here, the ligand takes an active role, triggering the conformational changes. By contrast, the conformational equilibrium model of allostery states that multiple functional states of the protein are in equilibrium. In this mechanism, the role of the ligand is subtler; it stabilizes a particular protein conformation by preferential binding. We are using unbiased all-atom molecular dynamics simulations of the GPCR rhodopsin to test the relevancy of these hypotheses. Rhodopsin, the visual photoreceptor, is a unique test case; both the active and inactive protein bind the same ligand, retinal. However, retinal adopts different conformations between the states, and the apo-protein, opsin, is outside the normal functional cycle. using simulations of four systems (apo- and holo-protein in the active and inactive states) we will evaluate the applicability of these allosteric models as well as describe how conserved regions of the protein are involved in activation.

2763-Plat
Adrenergic Receptors use Proton Conduction to Transduce Ligand Binding Energy into Activating Conformational Change
Jackson Chief Elk.
UC Santa Cruz, Santa Cruz, CA, USA.
It is proposed that proton conduction in class A G protein coupled receptors (GPCRs) assists in transducing orthosteric ligand binding energy into activating structural movement at their cytosolic domain. The binding site and cytosolic domain are 26 angstroms apart in the beta2 adrenergic receptor. How this action at a distance occurs is not fully understood. To investigate this problem, classical all-atom molecular dynamics simulations were used to simulate the behavior of the transmembrane (TM) domain under different orthosteric binding pocket conformations. The beta1 and beta2 adrenergic receptors were used as model GPCRs. Five different models were simulated for approximately 100ns each. Analysis of the simulations revealed that agonist (activating orthosteric ligand) binding causes a Grotthus-type water wire to form, connecting an aspartic acid and aspartate residue. Wire formation is the result of a bound agonist forcing changes in the bend angles of TM helices 4, 5, and 7. The changes in the bend angles form a hydrogen bond between a conserved aspartate in TM2 (D2.50) and the asparagine of the conserved NPxxY motif. Formation of this hydrogen bond results in a disruption of a solvent shell around D2.50, and as a consequence, a wire is formed. Water wires have been characterized to conduct protons in some membrane proteins Based on the simulation results, it is proposed that GPCR agonists drive proton conduction by reorienting internal waters in receptors through changes in TM helix bend angles. In this model, proton conduction in GPCRs occurs by a process similar to that in piezoelectric sensors. The forces applied by agonist binding reorients the internal dipoles of the receptor to make conduction energetically favorable and kinetically feasible. A mechanism is proposed to explain how proton conduction may activate receptors.

2764-Plat
Binding of Endogenous Cannabinoid Ligand 2-Arachidonoylglycerol to Cannabinoid Receptor CB2 via the Lipid Matrix
Tomohiro Kimura, Alexei Yeliseev, Klaus Gawrisch.
NIH/NIAAA, Bethesda, MD, USA.
Endogenous cannabinoid ligand, 2-arachidonoylglycerol (2-AG), binds to and activates cannabinoid type-2 receptor CB_2, controlling a variety of immune and hematopoietic cellular functions. Due to the high lipophilicity of cannabinoid ligands, there have been extensive discussions that after nonspecific incorporation into membrane, approach of the ligands to the binding pocket of CB2 may take place via lateral diffusion in lipid matrix. While previously reported microsecond-long molecular dynamics simulations showed molecular details in favor of this mechanism, no in-situ experimental evidence for the binding event has yet been made. Here we report on ^{2}H NMR results that show exchange of deuterated 2-AG-d_5 between the ligand binding site on CB_2 and the surrounding lipid matrix at physiological conditions. Purified, recombinant CB_2 was functionally reconstituted into liposomes containing four-fold molar excess of 2-AG-d_5, and its deuterium resonances measured over the temperature range of 5-20°C. When the membranes contained CB_2, the ligand resonances shifted upfield by 0.5 ppm and broadened significantly. Despite the excess amount of ligand, only one set of time-averaged resonances of the ligand was observed. The result suggests that 2-AG undergoes exchange between receptor-bound and free states on millisecond timescale. The resonance linewidth decreased with decreasing temperature as predicted from reduced exchange rates. The receptor binding was specific since only well-resolved signal of 2-AG in lipid matrix was observed when the binding pocket was blocked with a high affinity synthetic agonist CP-55,940. The experiments contrast to our previous experiments on CP-55,940 binding, where the free state of ^{2}H-labeled CP-55,940-d_6 in lipid matrix showed a highly-resolved signal under much slower exchange rate. The present work emphasizes the critical role of lipid matrix to provide a pathway and to regulate approach of lipophilic cannabinoid ligands to the receptor binding pocket.

2765-Plat
A Novel Mechanism for Acquiring GPCR Effector Selectivity
Liora Guy-David, Eitan Reuveny.
Weizmann institute of science, Rehovot, Israel.
The superfamily of the G-protein-coupled receptors (GPCRs) makes up the largest group of membrane receptors. When activated, by a large variety of ligands, they transduce an assortment of cellular responses via G-proteins cascade to affect cellular responses. Thus GPCR signaling must be a highly specific process regulated both temporally and spatially. One of the GPCR signaling regulators is G protein-coupled receptor kinase (GRK), responsible for GPCR signaling termination via the induction a cascade leading to of membrane internalization. Recently we identified an additional GRK-dependent regulatory mechanism that involves non-enzymatic mechanism to directly regulate effector function. One of the most notable effector of GPCR signaling is the activation of the G protein coupled potassium channels (GIRKs). These channels offer a direct short time scale monitoring of GPCR function. Interestingly, GIRK channel currents were found to be regulated by GRK2 in a GPCR-selective manner. In this project we investigated the mechanisms conferring specificity of receptors for GRK2 regulation of GIRK channels. In experiments involving both electrophysiology and fluorescent imaging, we have established that GRK2 has a different regulatory effect on GIRK channel currents when activated by different GPCRs. By using specific siRNA against the different Gβ subunits, we found that for various GPCRs-mediated GRK2 function, there is a differential dependence on a specific Gβ subunit. These finding suggest a governing pre-coupling between each GPCR to specific Gβ subunits. Additionally, we have found that G-protein subunits Gα and Gβ both affect the process of GRK2 recruitment to the membrane, but cannot dramatically compromise the specificity of this process for other GPCRs.
These findings may implicate of a new mechanism for acquiring specificities between GPCRs and downstream effectors and can further contribute to the understanding of non-enzymatic GRK2-dependnet modulation of various effectors.